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Investigation of the Relative Infectivity and Pathogenicity of Different Hepatitis C Virus Genotypes in Hemophiliacs

By Lisa M. Jarvis, Christopher A. Ludlam, Jennifer A. Ellender, László Nemes, Stephen P. Field, Ernest Song, Ampaiwan Chuansumrit, F. Eric Preston, and Peter Simmonds

To assess the relative infectivity and pathogenicity of variants of hepatitis C virus (HCV) genotypes, the distribution of genotypes in hemophilic patients who had been treated with nonvirally inactivated factor concentrates or cryoprecipitates prepared from local blood donors was compared with those found in the respective blood donor populations. Genotype frequencies differed markedly in the four countries investigated (Scotland, Hungary, South Africa, and

Thailand) but in each, the HCV genotype distributions in hemophiliacs and blood donors were similar. In addition, HCV genotypes in recipients of commercially manufactured concentrates were similar to those found in the US general population. These findings provide no evidence that HCV genotypes differ significantly from each other in replication rate, transmissibility, or infectivity.

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THE MAJORITY of hemophiliacs treated with pooled blood products before the mid 1980s have been infected with hepatitis C virus (HCV)¹⁻³ and it is generally accepted that HCV is responsible for most of the chronic liver disease observed in these patients. HCV can be classified into several distinct genotypes whose distributions show regional and ethnic variation,⁴⁻⁷ and considerable attention has recently focused on the possibility that biologic differences exist between different HCV types. In particular, certain HCV variants might differ in their relative pathogenicity, ie, in their infectivity, persistence, or replication. This possibility can be tested by comparing the relative frequencies of different genotypes in hemophiliacs that have been multiply exposed to nonvirally inactivated factor concentrates with those of the blood donor population from whose donations blood products were manufactured. In hemophiliacs, multiple exposure to HCV from long-term use of nonvirally inactivated concentrates has been shown to lead to multiple infections⁸ and possibly an increased likelihood of acquiring more pathogenic variants when compared to single-exposed individuals.

In this study, comparisons were made between hemophiliacs and blood donors from countries that differed considerably in geographical HCV genotype distribution. Differences in pathogenicity, if they exist between HCV variants, should be manifested by an increased or reduced frequency of detection of certain genotypes in the hemophiliacs compared with blood donors from whom blood products were manufactured in each of the countries.

A further comparison was made between HCV genotype distributions in hemophiliacs who have used commercially available factor concentrates and US blood donors⁷ and patients.^{9,10}

MATERIALS AND METHODS

Serum samples were separated on the day of collection and stored at -70°C. Virus RNA was extracted from stored serum samples by incubation with proteinase K/sodium dodecyl sulfate (SDS) as described previously.⁸ HCV RNA was reverse transcribed and amplified by polymerase chain reaction (PCR) using nested primers matching conserved regions in the 5' noncoding region (NCR).¹¹ Product DNAs were cleaved with restriction enzymes *HaeIII*/*Rsa* I and *Mva* I/*Hinf* I⁶ and the resultant (restriction fragment length polymorphism [RFLP]) patterns used to identify the different major genotypes of HCV. Subsequent digestion of product DNAs with restriction enzymes *Bst*UI and *Scr*FI allowed the identification of HCV subtypes 1a and 1b (*Bst*UI) and 2a, 2b, 3a and 3b (*Scr*FI).⁷ Restriction frag-

ments were separated by agarose gel electrophoresis using 4% Meta-phor agarose (FMC Bioproducts, Rockland, ME).^{7,8} Direct nucleotide sequencing of PCR products was performed as described previously.¹¹

RESULTS

HCV RNA from serum samples of 372 hemophiliacs from six different countries was amplified in the 5' NCR by reverse transcriptase-PCR (RT-PCR). Virus genotype was deduced by cleavage of product RNAs cleaved with restriction enzymes.^{7,8}

The HCV genotype distributions in cohorts that had been multiply transfused only with factor concentrates or cryoprecipitates manufactured locally were markedly different between countries. In Hungary all except one patient were infected with HCV genotype 1b (95%) (Fig 1A), whereas in Scottish hemophiliacs types 1a (44%) and 3a (31%) were the most prevalent with fewer examples of types 1b (11%), 2a (1%), 2b (5%), and 5 (1%) (Fig 1B). In the cohort of hemophiliacs from Johannesburg, South Africa, genotypes 1 through 5 were present, but genotype 5 (36%) was most frequent (Fig 1C). Hemophiliacs from Thailand were principally infected with type 3 (57%), although a significant proportion (15%) were infected with a series of novel genotypes

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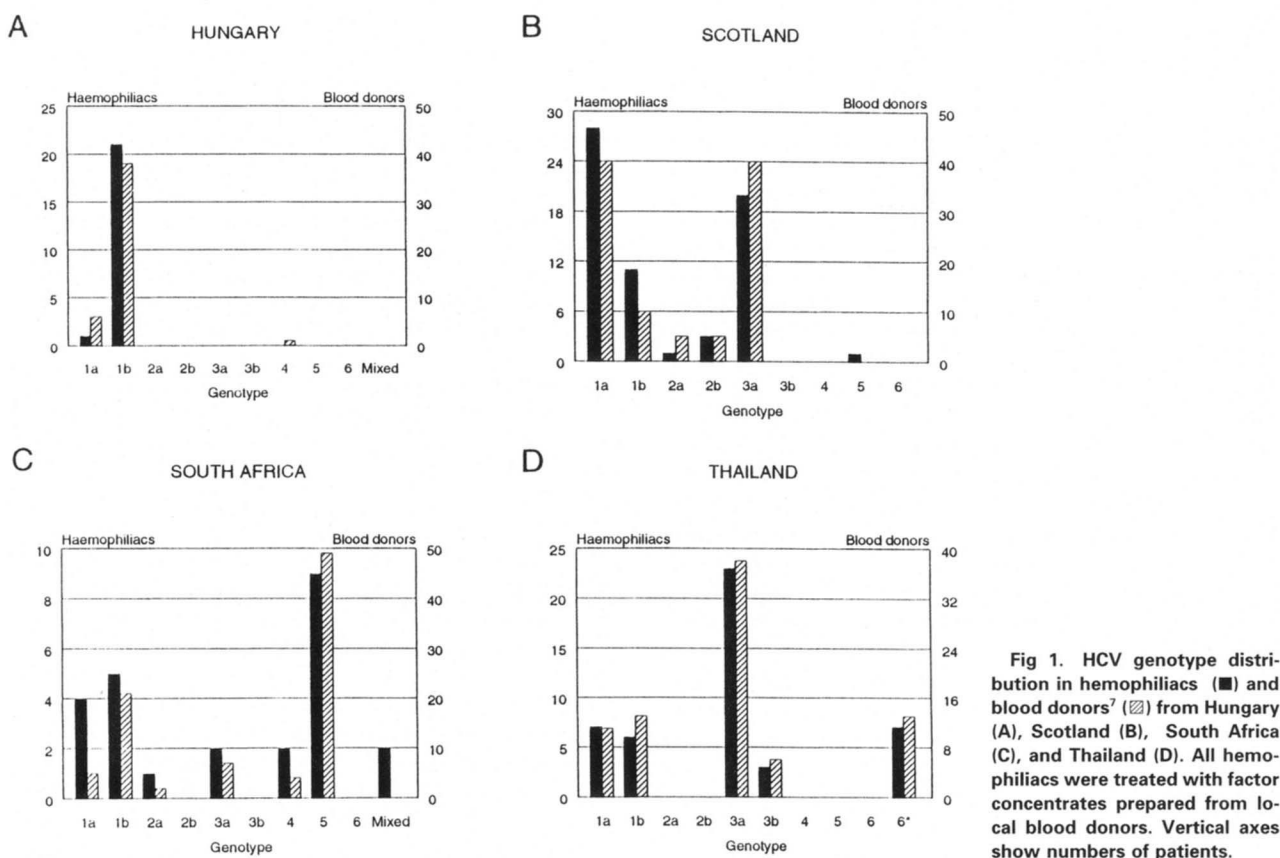


Fig 1. HCV genotype distribution in hemophiliacs (■) and blood donors⁷ (▨) from Hungary (A), Scotland (B), South Africa (C), and Thailand (D). All hemophiliacs were treated with factor concentrates prepared from local blood donors. Vertical axes show numbers of patients.

similar to those described in Vietnam,¹² Indonesia,¹³ and Thailand.^{14,15} These novel genotypes (with type 6) form a particular heterogeneous genotype distributed widely throughout Southeast Asia¹⁵ and in this study, have been classified together and labeled 6* (Fig 1D).

Comparisons of these distributions were made with those of the corresponding blood donor populations⁷ (Fig 1 A-D). For each country the observed HCV genotype frequencies in the hemophiliac cohorts closely mirrored the distinct patterns found in the corresponding blood donors. For example, the new HCV variants found in Thai hemophiliacs (described above as 6*) accounted for 15% of the infections in hemophiliacs and 16% in Thai blood donors. Similarly, 95% of the hemophiliacs in the Hungarian cohort were infected with genotype 1b as were 85% of the Hungarian blood donors surveyed. The predominant genotype in South African blood donors, equally common in black and white racial groups,⁷ was found to be type 5 (55%), and again this distribution was reflected in the corresponding hemophiliac cohort (36%).

In contrast, six cohorts of hemophiliacs, from the United Kingdom (Sheffield,¹⁶ Nottingham, Edinburgh), Germany, South Africa, and Hungary, which had received commercial factor concentrate (largely manufactured from US plasma), were most frequently infected with types 1a or 1b (36%, 27%). Genotype frequencies were similar in each country (data not shown) and resembled those found in US volunteer

blood donors⁷ and US patients with chronic HCV infection^{9,10} (Fig 2).

Infection with more than one genotype, as determined by RFLP analysis, was infrequently detected in both hemophili-

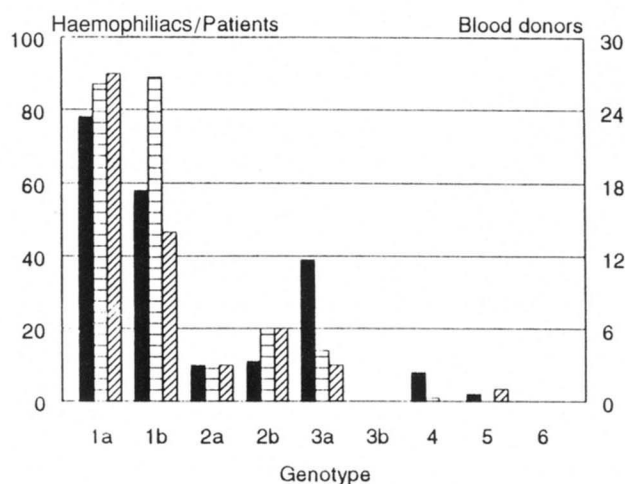


Fig 2. HCV genotype distribution in hemophiliacs treated with commercial factor concentrates (■), chronically infected US nonhemophiliac patients^{9,10} (▨), and volunteer US blood donors⁷ (□). Vertical axes show numbers of patients.

acs who received local (1%) and commercial concentrates (3%). However, this method will only detect the predominant variant and others genotypes may be present at low frequencies.

DISCUSSION

The absence of blood donor screening for HCV and use of nonvirally inactivated pooled clotting factor concentrates has exposed hemophiliacs to multiple episodes of HCV infection for a period of at least 10 years.¹⁻³ Despite the opportunity for more pathogenic variants of HCV to establish infection in hemophiliacs, we have found no evidence for over or underrepresentation of HCV genotypes in these patients compared with those to which they were exposed. The availability of data from several countries with different genotype distributions in the general population⁷ allowed comparisons of the relative pathogenicities of several genotypes to be compared (eg, types 1a and 3a in Scotland, types 1 and 5 in South Africa, and types 3a, 1, and 6* in Thailand).

Population differences in susceptibility to infection with different genotypes (such as variation in the frequency of different HLA alleles) could be ruled out as an explanation for the genotype differences observed because the genotype distribution in the hemophiliacs treated with commercial factor concentrates differed markedly from those found in the local blood donors. Indeed, for commercial factor users in each country (Germany, Hungary, South Africa, and United Kingdom) the same distribution of genotypes was observed as that of the US population (the main source of such blood products). Overall the similarity between hemophiliacs and blood donor genotypes strongly suggests that there are no significant differences between genotypes in replication rate, transmissibility, or susceptibility to immune clearance.

An alternative explanation for the similarity in genotype distribution between hemophiliacs and blood donors is that hemophiliacs became infected with the first genotype that they were exposed to and that this induced a protective immune response that prevented reinfection with other genotypes. However, there is no evidence for protective immunity in experimental animals reexposed to HCV,^{17,18} and repeated transfusion of HCV contaminated blood in thalassemic children led to symptomatic reinfection.¹⁹ Furthermore, direct evidence against protective immunity in the study group includes the detection of mixed infections in some hemophiliacs,^{16,20-22} and changes in the predominant circulating genotype with time.⁸ Indeed, in a longitudinal study of hemophiliacs in the Edinburgh cohort, we found genotype changes from type 1 (1a or 1b) to other genotypes as frequently as changes from other genotypes to type 1, providing further evidence for a lack of difference in pathogenicity between HCV variants.⁸

This study provides no evidence to support previous suggestions of a greater replicative capacity of HCV type 1^{9,10,23-26} because if this were the case, type 1 should be more frequently detected in multiply exposed individuals. Instead, this study is consistent with recent studies reporting similar virus loads for all genotypes, once the difference in sensitivity of assays such as branched DNA for different genotypes,

is considered.^{27,28} Similarly, measurement of virus load by PCR-based methods usually find no differences in virus levels between genotypes.^{29,30}

The proposed greater pathogenicity of type 1b^{31,32} would be expected to lead to the replacement of other genotypes from hemophiliacs with time, if it were associated with the development of more rapidly progressive liver disease. The failure of any one genotype to replace others in this study is inconsistent with such differences. It may be possible to argue that at least some of the previous studies showing a greater propensity for type 1b to cause more severe liver disease^{4,32,33} did not adequately control for compounding variables such as age, duration, and route of infection that may also show association with genotype and progression. In a large multivariate analysis of the development of cirrhosis in HCV-infected individuals, age was the only factor that was significant. Allowing for age, genotype showed no influence on the likelihood of development of cirrhosis.³⁴

Greater response rates to interferon (IFN) have been consistently found among individuals infected with HCV genotypes 2 and 3 compared to type 1b (or type 1) in Japan, Europe, and the United States.³⁵⁻³⁷ This difference remains even when other compounding factors such as patient age and pretreatment severity of disease have been accounted for.³⁸ In the Edinburgh hemophilia cohort, only hemophiliacs infected with genotype 3a showed any response to conventional 3 MU α -IFN three times per week, where six of seven normalized alanine transaminase levels and cleared viremia during treatment (compared with none of 18 type 1 infected individuals).³⁹ In view of the findings presented in this study, it appears unlikely that such genotype-specific differences in response can be attributed directly to pretreatment disease severity, variation in the propensity of certain variants to persist through a greater replication rate, or a decrease in the effectiveness of cytotoxic T cells to clear infection of the liver, although each of these may play an independent role in determining response. Instead it implies that there exist intrinsic differences between genotypes in their susceptibility to the antiviral action of IFN. The elucidation of the mechanism of resistance may provide an important step toward the development of more effective treatment for HCV infection in the future.

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